

Neutrophil CD64 Expression in Behçet's Disease

KEMAL ÜRETen, IHSAN ERTENLI, M. AKIF ÖZTÜRK, SEDAT KIRAZ, A. MESUT ONAT, MURAT TUNCER, HAMZA OKUR, ALI AKDOGAN, SULE APRAS, and MERAL ÇALGÜNERI

ABSTRACT. Objective. Hyperfunction of neutrophils is a characteristic finding in Behçet's disease (BD). Microbial agents have been proposed as causative agents in the disease flares. Fc gamma receptor 1 (CD64) is not normally expressed by neutrophils of healthy individuals, but is upregulated by these cells in response to microbial wall components and proinflammatory cytokines. The degree of polymorphonuclear leukocyte (PMN) CD64 expression is different in autoimmune diseases and systemic infectious diseases. We investigated PMN CD64 expression in patients with BD.

Methods. Thirty-seven patients with active BD (M/F: 18/19, mean age: 34.4 ± 9.7 yrs), 35 patients with inactive BD (M/F: 11/24, mean age: 35.9 ± 11.6 yrs), 27 patients with culture proven infections (M/F: 19/8, mean age: 54.4 ± 15.2 yrs), 31 healthy controls (M/F: 14/17, mean age: 37.7 ± 8.7 yrs), and 42 patients with active inflammatory disease (M/F: 13/29, mean age: 39.3 ± 14.9 yrs) were enrolled in this study. Flow cytometry was used to assess the prevalence of CD64-bearing PMN in whole blood samples.

Results. The prevalence of CD64-bearing PMN was significantly higher in patients with infectious disease (77.1 ± 18.4), inflammatory disease (37.1 ± 27.5), and active BD (48.9 ± 22.5) than in healthy controls (9.5 ± 7.8) or patients with inactive BD (12.9 ± 9.5). CD64 expression was similar in controls and patients with inactive BD. In the infectious disease group, expression of CD64 was significantly higher than in the active BD and active inflammatory disease groups, while there was no significant difference between the groups of patients with active BD and inflammatory disorders.

Conclusion. Neutrophil CD64 expression increases during exacerbation of BD. This increase appears to be a non-specific inflammatory response and does not reflect PMN activation triggered by a living microorganism. (J Rheumatol 2005;32:849–52)

Key Indexing Terms:

BEHÇET'S DISEASE DISEASE ACTIVATION CD64 NEUTROPHIL

Behçet's disease (BD) is a multisystem, chronic inflammatory disease of unknown etiology. The disease course is typically characterized by recurrent inflammatory attacks and periods of remission. Microbial agents have been proposed as causative agents in exacerbations of BD¹⁻³. In addition, neutrophil over-activation is a well known phenomenon, and there is evidence for the *in vivo* primed state of neutrophils in BD²⁻⁵. Fc receptors are found on the cell membrane of leukocytes and are functional in both innate and adaptive immune responses. Among the Fc receptors, Fcγ receptor 1 (CD64) is constitutively expressed on macrophages, monocytes, and eosinophils. However, CD64 is not normally expressed by polymorphonuclear leukocytes

(PMN) of healthy individuals, but is stored intracellularly and mobilized as a physiological response to microbial wall components, complement split products, and some proinflammatory cytokines⁶⁻⁹.

In a recent study, CD64 expression on the neutrophil surface was greater in autoimmune diseases than on neutrophils of patients with noninflammatory disease, and CD64 expression on PMN from patients with systemic infectious diseases was greater than on those from patients with non-inflammatory and inflammatory diseases⁹. Therefore, autoimmune disorders and systemic infections have distinct patterns of PMN CD64 expression.

Using flow cytometric analysis, we investigated whether the prevalence of PMN bearing CD64 was increased in patients with active compared to inactive BD and to patients with culture proven infections and healthy controls. Since PMN activation is not a specific feature of BD and may also be seen in other vasculitic syndromes and connective tissue disorders, patients with various inflammatory diseases other than BD were included in this study as disease controls.

MATERIALS AND METHODS

Patients. The study comprised 5 groups: 37 patients with active BD (M/F: 18/19, mean age: 34.4 ± 9.7 yrs), 35 patients with inactive BD (M/F: 11/24, mean age: 35.9 ± 11.6 yrs), 27 patients with culture proven infections (M/F: 19/8, mean age: 54.4 ± 5.2 yrs), 31 healthy controls from the hospital staff

From the Departments of Rheumatology and Pediatric Hematology, Hacettepe University School of Medicine; and the Department of Rheumatology, Gazi University School of Medicine, Ankara, Turkey.

K. Üreten, MD; I. Ertenli, MD, Department of Rheumatology, Hacettepe University; M.A. Öztürk, MD, Department of Rheumatology, Gazi University School of Medicine; S. Kiraz, MD; A.M. Onat, MD, Department of Rheumatology; M. Tuncer, MD; H. Okur, PhD, Department of Pediatric Hematology; A. Akdoğan, MD; S. Apras, MD; M. Çalgüneri, MD, Department of Rheumatology, Hacettepe University.

Address reprint requests to Dr. M.A. Öztürk, Ostim mahallesi, 89. sokak, AK-84 sitesi, A-2 blok, no: 8, Yenimahalle, Ankara, 06170 Turkey.

E-mail:makifozturk@yahoo.com

Accepted for publication December 20, 2004.

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2005. All rights reserved.

(M/F: 14/17, mean age: 37.7 ± 8.7 yrs), and 42 patients with active inflammatory disease (M/F: 13/29, mean age: 39.3 ± 14.9 yrs).

All of the patients with BD fulfilled the criteria of the International Study Group for BD¹⁰. Disease manifestations of the patients are summarized in Table 1. Patients with one or more of these features at the time of blood sampling were considered to have active disease. Seven patients had uveitis, 3 patients had vascular involvement, 6 patients had arthritis, 2 patients had genital ulcer, 2 patients had arthritis and oral ulcer, 3 patients had uveitis and oral ulcer, one patient had genital ulcer and oral ulcer, and 3 patients had erythema nodosum and oral ulcer during blood sampling. The remaining 10 patients in the active BD group had oral ulcer as the sole manifestation of clinical activation. Fourteen patients in the active BD group were newly diagnosed and were not receiving any medication during blood sampling. Only 3 patients in the inactive BD group were free of any medication. All of the remaining patients in both groups were using colchicine, low dose aspirin, and intramuscular benzathine penicillin, 1.2 million units every 3 weeks. Additionally, interferon (IFN) alpha, steroids, cyclosporine, and azathioprine were given to 6 patients, 4 patients, one patient, and 2 patients, respectively in the active BD group.

The infectious disease group consisted of 27 patients with culture proven infection of any kind. Urinary infection, wound infection, pneumonia, intra-abdominal abscess, and sepsis were documented in 12, 6, 3, 3, and 3 patients, respectively. Neutropenic patients were excluded from the study. All patients in this group were taking antibiotics at the time of blood sampling.

In the active inflammatory disease group, there were 12 patients with rheumatoid arthritis (RA) (29%), 9 patients with systemic lupus erythematosus, 9 patients with seronegative spondyloarthropathy (21%), 3 patients with sarcoidosis (7%), 3 patients with systemic vasculitis (7%), 4 patients with familial Mediterranean fever (10%), and 2 patients with systemic sclerosis (5%). All had active disease manifestations during blood sampling. As it has been shown that corticosteroids, disease modifying antirheumatic drugs (DMARD), and cytotoxic treatment have no significant impact on CD64 expression on neutrophils⁹, no patient was excluded for drug use.

We obtained approval from the ethics committee of Hacettepe University Medical School and informed consent for participation was obtained from all study patients.

Statistical analysis. Comparisons of the prevalence of CD64-bearing PMN between groups were performed with one-way analysis of variance (ANOVA). Statistically significant differences obtained from one-way ANOVA were further tested by the Tukey test for *post hoc* pairwise comparisons. The correlation between age and prevalence of CD64 expressing neutrophils was evaluated by the Pearson correlation test. Results were expressed as mean ± standard deviation (SD). A p value below 0.05 was considered statistically significant. The Statistical Package for Social Sciences (SPSS) version 11.0 was utilized for data analysis.

RESULTS

The mean age of patients in the infectious disease group was

Table 1. Clinical features of patients with BD. There was no significant difference regarding the frequency of any of these clinical manifestations between the active and inactive BD groups.

Clinical Manifestation	Active BD Group (n = 37)	Inactive BD Group (n = 35)	Total
Oral ulcer (%)	37 (100)	35 (100)	72 (100)
Genital ulcer (%)	33 (89.2)	29 (82.9)	62 (86.1)
Cutaneous findings (%)	17 (45.9)	19 (54.3)	36 (50)
Ocular manifestations (%)	18 (48.6)	19 (54.3)	37 (51.4)
Arthritis (%)	13 (35.1)	11 (31.4)	24 (33.3)
Vascular involvement (%)	7 (18.9)	5 (14.3)	12 (16.7)

significantly higher than the other groups ($p < 0.05$). There was no significant difference in age distribution among the remaining 4 groups. Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) values were significantly higher in patients with active BD, the infectious disease group, and the inflammatory disease group ($p < 0.05$). Patients with active and inactive BD were comparable regarding age and disease duration.

CD64-bearing PMN were significantly more prevalent in patients with infectious disease (77.1 ± 18.4), inflammatory disease (37.1 ± 27.5), and active BD (48.9 ± 22.5) than in healthy controls (9.5 ± 7.8) or patients with inactive BD ($p < 0.001$). CD64 expression was similar in healthy controls and patients with inactive BD (12.9 ± 9.5) ($p > 0.05$). In the infectious disease group the expression of the CD64 was significantly higher than in the active BD and active inflammatory disease groups ($p < 0.001$), while there was no significant difference between the groups of patients with active BD and active inflammatory disorders (Table 2, Figure 1). There was no correlation between age and prevalence of CD64 expressing neutrophils in any group. The effects of different clinical findings of BD on neutrophil CD64 expression were not investigated because of the limited number of patients with each clinical manifestation during blood sampling.

DISCUSSION

We have shown that the prevalence of CD64-bearing PMN in patients with active BD was significantly higher than in patients with BD in remission and healthy controls; this is comparable to patients with inflammatory disorders and significantly lower than patients with culture proven infections. The prevalence of CD64-bearing PMN in patients with inactive BD was similar to healthy controls.

The pathogenetic role of PMN in BD has been previously investigated. Significant neutrophil accumulation at active lesions of BD, including pustular folliculitis, pathergy reactions, and hypopyon is evident^{5,11}. Moreover, vari-

Table 2. The prevalence of CD64-bearing PMN in different study groups.

Group	Male/Female	Mean Age	CD64-Bearing Neutrophils (%)
Active BD	18/19	34.4 ± 9.7	48.9 ± 22.5 ^{a,b}
Inactive BD	11/24	35.9 ± 11.6	12.9 ± 9.5 ^{c,d}
Inflammatory disease	13/29	39.3 ± 14.9	37.1 ± 27.5
Documented infection	19/8	54.4 ± 15.2	77.1 ± 18.4 ^e
Healthy controls	14/17	37.7 ± 8.7	9.5 ± 7.8

^a $p < 0.001$ for active BD group vs inactive BD group, active BD group vs documented infection group, and active BD group vs healthy controls. ^b $p > 0.05$ for active BD group vs inflammatory disease group. ^c $p < 0.001$ for inactive BD group vs inflammatory disease group, and inactive BD group vs documented infection group. ^d $p > 0.05$ for inactive BD group vs healthy controls. ^e $p < 0.001$ for documented infection group and all other study groups.

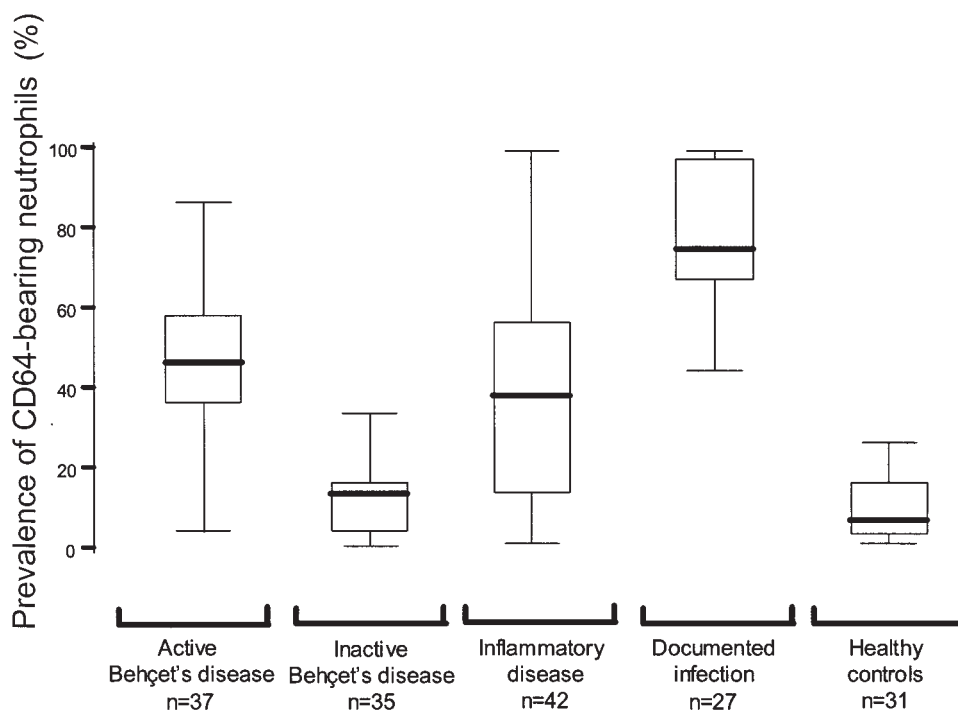


Figure 1. Prevalence of CD64 positive neutrophils in patients with active Behçet's disease, clinically inactive BD, active inflammatory disease, culture proven infections, and healthy controls.

ous *in vitro* experiments have found overactive neutrophils in patients with BD^{2,4,12,13}. Neutrophil surface antigen studies revealed increased expression of CD10 and CD14 in BD patients and patients with inflammatory arthropathies; moreover, increased CD14 expression was observed in active compared to inactive disease. Changes in surface molecules were more prominent in patients with sepsis⁴. These phenotypic changes reflect the state of activation of PMN in BD.

The clinical course of BD is undulating, with periods of activation and remission. Various microbial agents, mainly herpes simplex virus (HSV) and *Streptococcus sanguis* have been suggested as etiologic and/or triggering factors in BD pathogenesis¹⁻³. HSV DNA has been found in mucocutaneous lesions of patients with BD, and inoculation of HSV induced BD-like symptoms in mice^{2,14}. *S. sanguis* and antibodies against this microorganism are frequently found in oral mucosa and serum of BD patients, respectively^{1,2,15}. Increased oral manifestations after dental surgery are frequently observed in patients with BD. Moreover, prophylactic penicillin therapy is beneficial in controlling mucocutaneous manifestations and arthritis of BD^{16,17}. Microbial heat shock proteins (HSP) might be the common denominator of the proposed etiological factors in the pathogenesis of BD. Significant sequence homology exists between mammalian and microbial HSP. Infection induced stress upregulates HSP synthesis, and microbial HSP responsive T cells stimulate autoreactive T cells by cross reactivity mechanisms^{1,2,15,18}.

CD64 is considered an activation marker of neutrophils. Normally, CD64 is present on the surface of a few circulating PMN, but neutrophil CD64 expression rapidly increases as a response to microbial wall components, complement split products, and some proinflammatory cytokines including tumor necrosis factor alpha (TNF- α), IFN- γ , and interleukin 8 (IL-8)⁶⁻⁹. Hence, both bacterial infections and some autoimmune conditions cause upregulation of neutrophil CD64 expression^{9,19-21}. However, autoimmune disorders and systemic infections cause distinct patterns of PMN CD64 expression. In autoimmune disease PMN CD64 expression was greater than in noninflammatory disease, while in systemic infectious disease expression was greater than in noninflammatory and inflammatory disease⁹. Our results are comparable: CD64 expression was greater in active than in inactive BD and healthy controls, reflecting the state of neutrophil activation. However, CD64 expression was lower than in bacterial infections, and comparable to expression induced by other inflammatory disorders. Therefore, expression of those surface molecules reflects non-specific inflammation. Although previous reports suggested beneficial effects of prophylactic antibiotics in some clinical manifestations of BD^{16,17}, our results did not show PMN activation triggered by a living infectious microorganism. Increased levels of CD64 expressing PMN could be due to proinflammatory cytokines such as TNF- α , IFN- γ , and IL-8, which increase during BD exacerbations^{3,22-25}. Likewise, increases in CD10 and CD14 surface molecule expression on neutrophils were more prominent in patients

with sepsis than in patients with BD and patients with inflammatory arthropathies⁴.

Measuring activity of BD is difficult. There is currently no laboratory marker, including ESR and CRP, that correlates well with flares of BD^{26,27}. Moreover, both ESR and CRP increase in many infectious conditions. Quantitative measurement of neutrophil CD64 expression was suggested to distinguish between systemic infection and flares of autoimmune disease⁹. Likewise, flow cytometric determination of PMN CD64 expression warrants further investigation as a novel method to measure disease activity in BD and to discriminate between disease flare and infection. Owing to the limitations of our cross-sectional study, further studies with prospective followup are needed. It would also be interesting to evaluate the effects of different clinical manifestations of BD on PMN CD64 expression.

In conclusion, neutrophil CD64 expression increases during BD exacerbations. This increase appears to be a non-specific inflammatory response and does not reflect PMN activation triggered by a living microorganism. Therefore, the level of increase could distinguish between inflammation induced by flares of BD and microbial infections.

REFERENCES

1. Direskeneli H, Saruhan-Direskeneli G. The role of heat shock proteins in Behçet's disease. *Clin Exp Rheumatol* 2003;21 Suppl 30:S44-8.
2. Direskeneli H. Behçet's disease: infectious aetiology, new autoantigens, and HLA-B51. *Ann Rheum Dis* 2001;60:996-1002.
3. Gul A. Behçet's disease: an update on the pathogenesis. *Clin Exp Rheumatol* 2001;19 Suppl 24:S6-12.
4. Eksioglu-Demiralp E, Direskeneli H, Kibaroglu A, Yavuz S, Ergun T, Akoglu T. Neutrophil activation in Behçet's disease. *Clin Exp Rheumatol* 2001;19 Suppl 24:S19-24.
5. Sakane T, Takeno M, Suzuki N, Inaba G. Behçet's disease. *N Engl J Med* 1999;341:1284-91.
6. Simms HH, D'Amico R. Regulation of intracellular polymorphonuclear leukocyte Fc receptors by lipopolysaccharide. *Cell Immunol* 1994;157:525-41.
7. Hoffmeyer F, Witte K, Schmidt RE. The high-affinity Fc gamma RI on PMN: regulation of expression and signal transduction. *Immunology* 1997;92:544-52.
8. Qureshi SS, Lewis SM, Gant VA, Treacher D, Davis BH, Brown KA. Increased distribution and expression of CD64 on blood polymorphonuclear cells from patients with the systemic inflammatory response syndrome (SIRS). *Clin Exp Immunol* 2001;125:258-65.
9. Allen E, Bakke AC, Purtzer MZ, Deodhar A. Neutrophil CD64 expression: distinguishing acute inflammatory autoimmune disease from systemic infections. *Ann Rheum Dis* 2002;61:522-5.
10. International study group for Behçet's disease: Criteria for diagnosis of Behçet's disease. *Lancet* 1990;335:1078-80.
11. Kaklamani VG, Vaiopoulos G, Kaklamani PG. Behçet's Disease. *Semin Arthritis Rheum* 1998;27:197-217.
12. Pronai L, Ichikawa Y, Nakazawa H, Arimori S. Enhanced superoxide generation and the decreased superoxide scavenging activity of peripheral blood leucocytes in Behçet's disease: effects of colchicine. *Clin Exp Rheumatol* 1991;9:227-33.
13. Takeno M, Kariyone A, Yamashita N, et al. Excessive function of peripheral blood neutrophils from patients with Behçet's disease and from HLA-B51 transgenic mice. *Arthritis Rheum* 1995;38:426-33.
14. Sohn S, Lee ES, Bang D, Lee S. Behçet's disease-like symptoms induced by the Herpes simplex virus in ICR mice. *Eur J Dermatol* 1998;8:21-3.
15. Lehner T. The role of heat shock protein, microbial and autoimmune agents in the aetiology of Behçet's disease. *Int Rev Immunol* 1997;14:21-32.
16. Calguneri M, Ertenli I, Kiraz S, Erman M, Celik I. Effect of prophylactic benzathine penicillin on mucocutaneous symptoms of Behçet's disease. *Dermatology* 1996;192:125-8.
17. Calguneri M, Kiraz S, Ertenli I, Benekli M, Karaarslan Y, Celik I. The effect of prophylactic penicillin treatment on the course of arthritis episodes in patients with Behçet's disease. A randomized clinical trial. *Arthritis Rheum* 1996;39:2062-5.
18. Direskeneli H, Eksioglu-Demiralp E, Yavuz S, et al. T cell responses to 60/65 kDa heat shock protein derived peptides in Turkish patients with Behçet's disease. *J Rheumatol* 2000;27:708-13.
19. Herra CM, Keane CT, Whelan A. Increased expression of Fc gamma receptors on neutrophils and monocytes may reflect ongoing bacterial infection. *J Med Microbiol* 1996;44:135-40.
20. Leino L, Sorvajarvi K, Katajisto J, et al. Febrile infection changes the expression of IgG Fc receptors and complement receptors in human neutrophils in vivo. *Clin Exp Immunol* 1997;107:37-43.
21. Quayle JA, Watson F, Bucknall RC, Edwards SW. Neutrophils from the synovial fluid of patients with rheumatoid arthritis express the high affinity immunoglobulin G receptor, Fc gamma RI (CD64): role of immune complexes and cytokines in induction of receptor expression. *Immunology* 1997;91:266-73.
22. Sayinalp N, Ozcebe OI, Ozdemir O, Haznedaroglu IC, Dundar S, Kirazli S. Cytokines in Behçet's disease. *J Rheumatol* 1996;23:321-2.
23. Evereklioglu C, Er H, Turkoz Y, Cekmen M. Serum levels of TNF- α , sIL-2R, IL-6, and IL-8 are increased and associated with elevated lipid peroxidation in patients with Behçet's disease. *Mediators Inflamm* 2002;11:87-93.
24. Zouboulis CC, Katsantonis J, Ketteler R, et al. Adamantiades- Behçet's disease: interleukin-8 is increased in serum of patients with active oral and neurological manifestations and is secreted by small vessel endothelial cells. *Arch Dermatol Res* 2000;292:279-84.
25. Hamzaoui K, Hamzaoui A, Guemira F, Bessiod M, Hamza M, Ayed K. Cytokine profile in Behçet's disease patients. Relationship with disease activity. *Scand J Rheumatol* 2002;31:205-10.
26. Bhakta BB, Brennan P, James TE, Chamberlain MA, Noble BA, Silman AJ. Behçet's disease: evaluation of a new instrument to measure clinical activity. *Rheumatology Oxford* 1999;38:728-33.
27. Muftuoglu AU, Yazici H, Yurdakul S, et al. Behçet's disease. Relation of serum C-reactive protein and erythrocyte sedimentation rates to disease activity. *Int J Dermatol* 1986;25:235-9.